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RESEARCH ARTICLE

Anti-implantational Effects of *Catharanthus roseus* Leaf Extract in Swiss Albino Mice.

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Abstract

Petroleum ether extract of leaves of "Sadabahar" *Catharanthus roseus* plant was subjected intramuscularly (im) to mated females from day 7 to 9 postcoitum (pc) at the doses of 5 and 10 mg/day/mouse. At autopsy on day 12 pc 100% inhibition of pregnancy was observed. When the extract at the same dose regimen was administered to adult males for 20 days, antifertility effects were manifested. If such treated males were mated with normal parous females, successful mating could be achieved without any loss of libido but in the females approximately 80 to 100 percent dose dependent decrease in the number of implantations was recorded. The glycogen and cholesterol content of the uterus and testis of the treated females and males respectively were also markedly altered. Thus, it is concluded that the antifertility effects are due to anti-implantational properties of the extract.

Keywords

Catharanthus leaves, implantation, Petroleum ether, postcoitum (pc)

Introduction

Many herbal remedies are traditionally used as contraceptives (to prevent ovulation or fertilization), abortifacients (to prevent implantation), emmenagogues (to stimulate uterine flow) or oxytocics (to stimulate uterine contractions, particularly to promote labour) (Ritchie, 2001). Plant products have attracted the attention of many scientists as a primary source of naturally occurring fertility regulating agents because of their little or no side effects (Duragkar *et al*, 2007). The use of plant extract and fractions for antifertility activity especially for prevention/ intervention of pregnancy have been in practice since ancient times in India (Norman, 1975). In 1988, the Natural Products Alert database had recorded 4,410 plants used as emmenagogues, 2,630 as abortives and 1,249 as contraceptives (Ritchie, 2001). A literature survey for period of 25 years (1980-2005) revealed that there are about 105 plants which possess antifertility activity in males (Gupta and Sharma, 2006). Though many indigenous plants are known to posses antifertility properties, only a few have been investigated in detail for their antinidational effect (Dhir *et al*,1987; Jacob *et al*,1988).Failure of nidation has also been reported in female rodents mated with oestrogen treated males; the effect conjecturally results from either transport of estrogen in the semen to the vicinity of the uterus causing expulsion and degeneration of fertilized eggs (Ericsson and Baker,1966; Scubliansky and Woltz,1971; Vyas and Jacob,1986) .The present study aims to investigate the anti-implantational properties of the leaves of a common periwinkle plant(*Catharanthus roseus* ; Family Apocyanaceae) in the mouse.

Material and Methods

Colony bred adult male and female mice (18-22 gm) of proven fertility, maintained under laboratory conditions on the balanced diet and water *adlibitum*, were used in this study.

The leaves of *Catharanthus roseus* were locally obtained from agricultural farms and nurseries near Jaipur. The leaves were dried, ground and the leaf extract was prepared by Soxhlet's extraction procedure using Petroleum ether (b.p.60°C-80°C) as the solvent. The extract was concentrated and air dried under vacuum. The daily dose of leaf extract (5 mg or 10 mg) was dissolved in 0.1 ml olive oil and injected intramuscularly. A set of suitable vehicle treated control was also maintained.

For convenience of description the present experimentation was divided into 3 categories and the regimen followed for each has been in that particular category.

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Postcoital antifertility effect

For this test parous non-pregnant females were mated with males of proven fertility. Mating was confirmed by the presence of vaginal plug and/or spermatozoa in the vaginal smear. The day of mating was taken as day 0.To these mated females the petroleum ether extract of *Catharanthus roseus* leaves was injected (im) at a dose of 5 and 10 mg/day/mouse from 7 to 9 pc. Control and experimental animals were autopsied on day 12 pc and the number of corpora lutea , implantation sites, if any, were counted in each uterine horn.

Effect on nidation after mating females with *Catharanthus roseus* treated males

Adult males (4 to 6 weeks old) of proven fertility were injected (im) with the Petroleum ether leaf extract at a dose of 5 and 10 mg/day/mouse every alternate day for 20 days. These males were mated with parous females and mating in each case was confirmed by the presence of vaginal plug and /or spermatozoa in the vaginal smear. The day of mating was taken as day 0. The mated females were autopsied on day 15 pc and the number of corpora lutea and implantation sites, if any, were counted in each uterine horn.

Effect on cholesterol and glycogen content of uterus and testis

From the above set of treated males, 2-3 animals from each group were autopsied 24 hrs after the last injection. Similarly, the females were sacrificed at the day 12 pc along with a set of suitable controls. The testis and uterus were dissected out respectively, trimmed of adherent tissue and weighed to the nearest mg. Biochemical estimations of cholesterol and glycogen content of uterus and testis were done by the method of Liebermann and Burchard (1952) and Rex Montgomery (1957) methods respectively.

Results and Discussion

Postcoital antifertility effect

Table 1 shows the anti-implantation activity of *Catharanthus roseus* leaf extract in mice. The total number of corpora lutea in control females ranged from 10 to 12 while in the experimental groups it ranged from 12 to 15. Intramuscular injection of the leaf extract of *Catharanthus roseus* effectively interrupted pregnancy at both the tried doses: 5mg and 10 mg/day/mouse. The females of the treated group did not show any trace of implantation sites. However, one animal at the low dose regimen of 5 mg did show the presence of five resorbing fetuses.

Effect on nidation after mating females with *Catharanthus roseus* leaf extract treated males

Mating proestrus females with 20 day treated males resulted in approximately 80 percent inhibition of nidation at the dose of 5 mg/day/mouse while total interruptive effect was obtained at the dose of 10 mg/day/mouse (Table 2).

Effect on cholesterol and glycogen content of uterus and testis

The glycogen content of the uterus and testis of treated animals at the dose regimen of 5 or 10 mg/day/mouse exhibited a highly significant decline. The cholesterol content of the uterus decreased significantly at 5 mg dose while this decrease was highly significant in the 10 mg treated mice. While the cholesterol content of the testis of treated males showed a highly significant increase at the 10 mg/day dose; the increase in cholesterol was also statistically significant at the 5 mg/day dose regimen (Table 3).

Table 1. Postcoital anti-implantational efficacy of petroleum ether extract of *Catharanthus roseus* leaves in mice

Treatment :Day 7 to 9 postcoitum	Autopsy on day 15 postcoitum		
	Number of corpora lutea	Number of Implantation sites	Percentage of implantation sites
Control	59	50	84.74
Experimental (mg/day/mouse)			
5	64	0	0*
10	60	0	0

*Except in 2 animals which exhibited 5 dead fetuses/12 corpora lutea

Table 2. The effect on nidation of females mated with petroleum ether extract of *Catharanthus roseus* extract treated males for 20 days

Males Treatment for 20 days	Females Autopsy on day 15 pc		
	Number of corpora lutea	Number of implantation sites	Percentage of implantation sites
Control	64	55	85.94
Experimental dose (mg/day/mouse)			
5	53	0	0*
10	58	0	0

*Except in one female in which six resorbing fetuses were observed/12 corpora lutea

Table 3. Effect of *Catharanthus roseus* leaf extract on the glycogen and cholesterol content of the reproductive organs in treated males and females

Group	Glycogen Mean±S.EM		Cholesterol Mean±S.EM	
	Uterus	Testis	Uterus	Testis
Control	8.3± 0.6	3.3± 0.0005	11.08± 0.7	4.6± 0.6
Experimental dose				
5 mg/day/mouse	5.1± 0.5***	1.4± 0.2***	6.4±0.7***	6.5± 0.8*
10 mg/day/mouse	5.4± 1.04**	0.87± 0.003***	8.4±2.2*	5.2±0.3*

Significance in relation to control * $p<0.05$, ** $p<0.01$, *** $p<0.001$

From the results obtained in the present investigation, it is evident that the petroleum ether leaf extract of *Catharanthus roseus* possesses distinct antifertility efficacy in the female mouse by inhibiting nidation. The fertility of the male mouse is also affected if the test substance is chronically administered for 20 days and if such treated males are treated with parous proestrus females then nidation is affected. In this effect the leaf extract of *Catharanthus roseus* simulates a number of other plant substances,e.g., *Malvaviscus conzatti* flower (Joshi *et al* 1981),'Champa' leaf (Vyas and Jacob,1986) and *Daucus carota* and *Nigella sativa* seeds (Vyas and Jacob,1989). Besides evaluation of the antifertility efficacy, an effort was also made of the antifertility effect of *Catharanthus roseus* leaf by examining the glycogen and cholesterol content of the uterus and the testis. A decrease is obtained in the uterine content of the tested biochemical parameter but in the treated male the testicular cholesterol increases while the glycogen content decreases.

The antifertility effect and the alterations in the biochemical content of the uterus and testis of the treated mice can conjecturally be co-related. Decreased glycogen level is in accordance with the view of Davis and Firlit (1965) who speculated that protein synthesis in spermatogenic cells is dependent upon glucose. A marked decrease in the glycogen content could effect protein synthesis and thus subsequently inhibit spermatogenesis. Thus a relationship could be established between glucose and protein synthesis in certain cell types of rat testes (Davis *et al*, 1964) and enzymes responsible for this process are very sensitive to the presence of glucose *in vitro*. Reduction in testicular glycogen may also be due to decreased number of post meiotic cells which are the sites of glucose metabolism (Gunaga *et al*, 1972). Decrease in uterine glycogen signifies the alteration in the activities such as steroidogenesis etc.

The requirement of cholesterol for normal hormonal activity of the testis was emphasized by Terroine (1965) Changes in the testicular cholesterol levels are considered to be important, since it is implicated in the inhibition/ stimulation of spermatogenesis (Hall *et al*, 1969).The increase in cholesterol values of testes implies inhibition of androgenesis and impairment of spermatogenesis. Since androgen level is related to fertility and sperm output, the accumulation of cholesterol in testis constitutes a direct evidence for

androgen-antagonistic action that leads to infertility. The dose must have interfered with cholesterol utilization by steroidogenic cells, which explains decreased cholesterol in uterine tissue, as has been reviewed in a recent study.

Ikonen (2008) emphasized that the contribution of *de novo* cholesterol synthesis versus intake has been estimated as a ratio of 70:30. Therefore, interference in *de novo* synthesis could be primary source of observed changes in cholesterol level in the uterine tissue.

However, it shall be premature to draw any definite conclusions at the juncture excepting that the antifertility effect at least in the male could possibly be due to the inherent antiandrogenic testicular inhibitory effect as evidenced by its increased cholesterol content and decreased glycogen content(Moore and Bedford 1979).

Further work in the direction of obtaining an insight on the mode of action of these interesting plant substances is in progress.

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